cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), wherein the Cys of each monomer is disulfide bonded to an additional extraneous Cys, in admixture with a pharmaceutically acceptable vehicle.

15. (Amended) The composition of claim 14 wherein the Cys residue in at least one of said first monomer or said second monomers is part of a peptide of 2-5 amino acids.

17. (Amended) The composition of claim 16 wherein each monomer is disulfide bonded through the Cys residue, to a glutathione moiety.

REMARKS

In response to the Office Action mailed July 3, 2002, Applicant respectfully requests the Examiner to reconsider the above-captioned application in view of the foregoing amendments and the following comments. A one-month extension of time is requested for this amendment and response. As a result of the amendments listed above, Claims 1-74 are pending, Claims 35-74 are withdrawn from consideration. Claims 4, 12, 22-24, and 26-34 have been cancelled without prejudice. Claims 1, 2, 13, 14, 15, and 17 have been amended.

The specific changes to the specification and the amended claims are shown on a separate set of pages attached hereto and entitled VERSION WITH MARKINGS TO SHOW CHANGES MADE, which follows the signature page of this Amendment. On this set of pages, the insertions are underlined while the ~~deletions are stricken through~~.

Telephonic Interview

Applicants thank the Examiner for the courtesy shown during the telephonic interview conducted on October 8, 2002.

Formal Matters

The PTO has raised a number of formal matters in the pending Office Action. Applicants thank the Examiner for her offer to consider the Information Disclosure Statement filed January 9, 2001. The non-patent references requested by the Examiner are enclosed with the present response. No additional form PTO-1449 will be submitted.

The PTO has objected to claims 28-34 as allegedly encompassing multiple, patentably distinct inventions. Without acquiescing to the objection, Applicants have cancelled claims 28-34, without prejudice.

The PTO has objected to the title of the application as not being descriptive. Applicants have amended the title to better reflect the subject matter of the present invention.

The PTO has objected to the disclosure for containing informalities. The specification has been amended to correct the typographical error noted in the Office Action.

Claims 1-34 particularly and distinctly recite the subject matter that applicants regard as their invention.

The PTO has rejected claims 1-34 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Specifically, the PTO rejected claims 1, 14, and 28 and suggested claim language to clarify the subject matter of the claims. The PTO also rejected claims 4 and 17. Applicants have amended claims 1, 14, and 17 in accordance with the suggestions of the PTO in the Office Action. Applicants have cancelled claims 4 and 28, rendering the rejection of these claims moot. In view of the amendments to the claims, Applicants submit that the present rejection of claims 1-34 be withdrawn.

The specification of the present application enables the full scope of Claims 1-34.

The PTO has rejected claims 1-34 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. "To be enabling, the specification of a patent must teach those skilled in the art to make and use the full scope of the claimed invention without 'undue experimentation' ... Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples." *See In re Wright*, 999 F.2d 1557 (Fed. Cir. 1993). The PTO has alleged that the specification does not enable a) dimers that comprise monomers of longer forms of VEGF; b) dimers comprising monomers shorter than $VEGF_{121}$; or c) dimers in the form of homogenous, 75% pure, 85% pure, or 95% pure preparations. Applicants address each of these points below.

The specification enables VEGF dimers that comprise monomers of longer forms of VEGF.

The PTO has rejected Claims 1-34 for allegedly lacking enablement. The human *VEGF-A* gene comprises eight exons separated by seven introns. Alternative splicing of the *VEGF-A* gene results in the generation of at least seven splice variants (isoforms), each containing 121, 145, 148, 165, 183, 189, and 206 amino acids respectively. Specification at Figure 2. The Cys-

116 residue of $VEGF_{121}$ is encoded in Exon 8. Specification, p. 14, ll. 15-16. As shown in Figure 2, every VEGF isoform except the $VEGF_{148}$ has amino acids encoded by Exon 8. Thus, every VEGF variants regardless of length, that possesses the amino acids encoded by exon 8 (*e.g.*, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$) each possess a Cys residue that corresponds to position 116 of SEQ ID NO: 1. Because one of ordinary skill in the art would know how make a VEGF protein contain the amino acids encoded by exon 8, the pending claims are enabled.

The PTO additionally alleges that Applicants discuss particular functional characteristics possessed by the claimed VEGF variants. The PTO then alleges that it would not be predictable that VEGF isoforms longer than 121 amino acids would have these characteristics. Applicants respectfully note that the question of enablement turns on whether the claimed invention is supported by an enabling disclosure. Because the characteristics of increased stability are not recited in the pending claims, a rejection for an alleged lack of enablement for such features is improper and should be withdrawn.

The specification enables VEGF dimers that comprise shorter forms of VEGF monomers.

The PTO has allegedly that Claims 1-34 lack enablement for monomers shorter than $VEGF_{121}$. The test for enablement is whether one of ordinary skill in the art could practice the full scope of the claimed invention without undue experimentation. "That some experimentation is necessary does not preclude enablement; the amount of experimentation, however, must not be unduly extensive." *Atlas Power Co. v. E.I. Du Pont De Nemorus & Co.*, 750 F.2d 1569, 1573 (citing *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557, 220 U.S.P.Q. (BNA) 303, 316 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851, 105 S. Ct. 172, 83 L. Ed. 2d 107, 53 U.S.L.W. 3239 (1984)).

The PTO noted in the office action that the specification discloses $VEGF_{121}$ monomers produced in two forms, one possessing 120 amino acids and the other possessing 121 amino acids. Specification at page 15, lines 9-28; page 29, line 34 to page 30, line 4. These truncated proteins were produced in Chinese hamster ovary cells (CHO). In contrast, Examples 2 and 3 discuss the expression of $VEGF_{121}$ proteins in *E. coli* and *P. pastoris*, in which no truncations were discussed. Accordingly, Applicants submit that the full scope of the pending claims is

enabled because the specification teaches the production of full length VEGF protein in cell lines other than CHO cells without truncations.

Applicants also note that expression of a $VEGF_{121}$ coding sequence in CHO cells produced a mixture of $VEGF_{121}$ variants comprising proteins of 120 and 121 amino acids in length. Accordingly, even if CHO cells were the only cells available with which to express a recombinant VEGF variant, one of ordinary skill in the art could use the CHO cells and purify out the proteins of interest from any truncated products.

In light of the remarks above Applicants submit that the pending claims are supported by an enabling specification. As such, Applicants request that the present rejection be withdrawn.

The specification enables compositions of VEGF dimers that are homogenous, 75% pure, 85% pure, or 95% pure preparations.

Applicants have cancelled Claims 26-34, without prejudice or admission regarding the present rejection. Nevertheless, cancellation of Claims 26-34 renders the present rejection moot.

The pending claims are novel over Tischer.

The PTO rejected Claims 1-25 and 28-30 as allegedly being anticipated by Tischer, et al., U.S. Patent No. 5,194,596 (Tischer). To anticipate a claim, a reference must teach each and every limitation of the claimed invention. Applicants have amended Claims 1 and 14 to recite VEGF variants that have a glycosylation site at or corresponding to positions 75-77 of SEQ ID NO: 1 that has been eliminated. Tischer does not teach such a VEGF variant. Because Tischer fails to teach each and every limitation of the claimed invention, the pending claims are novel over Tischer. Accordingly, the anticipation rejection should be withdrawn.

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, amendments, the reasons therefor, and arguments in support of the patentability of the pending claim set are presented above. Any claim amendments which are not specifically discussed in the above remarks are made in order to improve the clarity of claim language, to correct grammatical mistakes or ambiguities, and to otherwise improve the capacity of the claims to particularly and distinctly point out the invention to those of skill in the art. In light of the above amendments and remarks, reconsideration and withdrawal

of the outstanding rejections is specifically requested. If the Examiner finds any remaining impediment to the prompt allowance of these claims that could be clarified with a telephone conference, the Examiner is respectfully requested to initiate the same with the undersigned.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 1 NOV 2002

By: _____

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Below, insertions are underlined while the ~~deletions are stricken through~~.

IN THE SPECIFICATION:

On pages 1 and 48, please amend the title to read as follows: "VASCULAR ENDOTHELIAL GROWTH FACTOR DIMER VARIANTS"

Please amend the paragraph 18, line 33 to page 19, line 11 as follows:

In *E. coli*, the $VEGF_{121}$ monomers typically accumulate in the form of inclusion bodies, and need to be solubilized, refolded, dimerized and purified. Methods for the recovery and refolding of VEGF isoforms from *E. coli* are known in the art. For example, refolding of certain VEGF isoforms following recombinant expression in *E. coli* is described in Christinger *et al., Prot. Struc. Func. Genet. supra* (1996); Keyt *et al., J. Biol. Chem.* 271:7788-7795 (1996); Cao *et al., J. Biol. Chem.* 271:3154-3162 (1996); Siemeister *et al., Biochem. Biophys. Res. Commun. 222*:249-255 (1996); and PCT Publication WO 96/06641. In a particularly preferred embodiment of the present invention refolding is performed in the simultaneous presence of cysteine and cystine in the refolding buffer. By adjusting the amounts and mutual ratio of cysteine and cystine, one can produce the desired mix of VEGF dimers. The latter embodiment is specifically illustrated in the Examples below. In a preferred embodiment, free cysteine used in the refolding step is added in molar excess from about 4-fold to about 40-fold over the cysteines present in the VEGF polypeptide. More preferably, the free cysteine is used in from about 4-fold to about 20-fold, even more preferably from about 4-fold to about 10-fold, most preferably about 10-fold molar excess over the cysteines present in the VEGF polypeptide. The cysteine to cystine molar ratio generally is between about 2:1 and 20:1, preferably between about 2:1 and 10:1, more preferably between about 2:1 and 5:1, most preferably about 4:1 and 5:1.

IN THE CLAIMS:

Please cancel claims 4, 12, 22, 23, 24, 26-34 without prejudice.

Please amend claims 1, 2, 13, 14, 15, and 17 as follows:

1. (Amended) A vascular endothelial growth factor (VEGF) variant dimer consisting of a first monomer and a second monomer, ~~each comprising at least amino acids 11 to 116 of SEQ ID NO: 1, or~~ comprising an amino acid sequence having at least about 90% sequence

identity with amino acids 11 to 116 of SEQ ID NO: 1, retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), wherein the Cys ~~-116~~ of each monomer is disulfide-bonded to an additional extraneous Cys, and wherein at least one monomer possesses a glycosylation site at or corresponding to positions 75-77 of SEQ ID NO: 1 that has been eliminated.

2. (Amended) The VEGF dimer of claim 1 wherein the Cys residue in at least one of said first monomer or ~~and~~ second monomers, or both, is disulfide-bonded to a ~~said additional Cys is part of~~ a peptide of 2-5 amino acids.

13. (Amended) The VEGF dimer of claim 1 wherein ~~at least one of~~ said first and second monomers ~~is~~ are unglycosylated.

14. (Amended) A composition comprising a vascular endothelial growth factor (VEGF) dimer consisting of a first monomer and a second monomer, each monomer comprising ~~at least amino acids 11 to 116 of SEQ ID NO: 1, or comprising~~ an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, wherein at least one monomer possesses a glycosylation site at or corresponding to positions 75-77 of SEQ ID NO: 1 that has been eliminated, and retaining a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), wherein the Cys~~-116~~ of each monomer is disulfide bonded to an additional extraneous Cys, in admixture with a pharmaceutically acceptable vehicle.

15. (Amended) The composition of claim 14 wherein the Cys residue in at least one of said first monomer or ~~and~~ said second monomers ~~said additional Cys~~ is part of a peptide of 2-5 amino acids.

17. (Amended) The composition of claim 16 wherein each monomer is disulfide bonded~~,~~ through ~~a~~ the Cys residue~~,~~ to a glutathione moiety.

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